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Data generated from three quantitative mass spectral methods for the analysis of trivalent influenza vaccine antigens are compared



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ABSTRACT

Herein we present the data necessary for generation of alternative means to produce equimolar mixtures of peptides (“Design and Expression of a QconCAT Protein to Validate Hi3 Protein Quantification of Influenza Vaccine Antigens” (D.G.S. Smith, G. Gingras, Y. Aubin, T.D. Cyr, 2016) [1]), such as QconCAT (“Trends in QconCATs for targeted proteomics” (J. Chen, I.V. Turko, 2014) [2], “Natural flanking sequences for peptides included in a quantification concatamer internal standard” (C.S. Cheung, K.W. Anderson, M. Wang, I.V. Turko, 2015) [3]) and SpikeTides versus the label free Hi3 approach. The experimental design and the interpretation of results are discussed in the original article [1].

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Specifications Table

Subject area	Chemistry
More specific subject area	Absolute quantitative proteome analysis
Type of data	Gene sequences, tables, graphs, links, fasta protein database
How data was acquired	Mass spectroscopy, QTOF (Waters Synapt HDMS system (Milford, MA, USA))

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Data format	<i>Analyzed</i>
Experimental factors	<i>The samples were digested with a higher than usual ratios of trypsin/substrate, source voltages optimized to reduce in-source fragmentation</i>
Experimental features	<i>The peptides were analyzed on a using a reversed phase (BEH130 C18) column with data independent MSMS then data analysis using PLGS 3.0 Waters Ltd.)</i>
Data source location	<i>n/a</i>
Data accessibility	<i>Data is within this article</i>

Value of the data

- Excellent reproducibility of peptide concentration data produced by the analysis of tryptic digestions is the necessary for method comparisons. The costs associated with a particular approach in terms of reagents and time may be a pivotal factor.
 - The Hi3 method is a very valuable approach in cases of relatively simple proteomes. The speed of method development for core laboratories can be critical. The Hi3 method is relatively straightforward, once reproducible results are obtained.
 - The QConCAT design is very elegant but there are critical issues, such as the protein construct solubility and stability, which require careful attention to obtain optimal results. The database attached is necessary for analysis of the mass spectral results.
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1. Data

The data are compiled in the bar graphs for the quantitative proteome analyses of trivalent influenza vaccines and standards using three methods: Hi3, QconCAT and synthetic peptides.

Databases contained either the customized QconCAT protein sequence or the full sequences of the proteins represented therein, along with trypsin, several human keratins (cRAP from <http://www.thegpm.org/crap/>) and, since the viruses are grown in chicken eggs, the entire chicken (*Gallus gallus*) proteome (ftp://ftp.ensembl.org/pub/current_fasta/gallus_gallus/pep/) as well as selected full length influenza proteins (mostly from GISAID <http://platform.gisaid.org/epi3/frontend#4f5b25>, and the World Health Organization <http://www.who.int/influenza/vaccines/virus/en/>).

2. Experimental design, materials and methods

Data was obtained by the comparison of mass spectral signal from the three most intense fully tryptic peptides identified from the samples versus the internal standard protein. Experiments were designed to compare the mass spectral signal strengths from equimolar tryptic peptides identified by the Hi3 method by five different QconCAT designs as well as from synthetic peptides. The samples analyzed were commercial trivalent influenza vaccines as well as monovalent influenza reference standards. The mass spectra were obtained by reversed phase separation using a C18 UPLC column, Waters nanoAcquity UPLC, directly coupled to a Waters Synapt HDMS mass spectrometer. The mass spectrometer was programmed to carry out data-independent MSMS and incorporated a lock spray of glu-fibrinopeptide. The data was processed using Protein Lynx Global Server 3.0 for the identification of the three most intense peptides from a given protein identified in a custom database which has been attached. The intensities from all charge states of a peptide are included in the software calculations; however, the intensities resulting from in-source fragmentation and modified peptides must be added manually.

2.1. Codon optimized gene sequences

2.1.1. QconCAT 1

CATATGGATGACGATGATAAAGTGGTGAACGAAGTACCGAATTCGGGAACTGGGCGAATACGGCTTCCA-
 GAATGCACTGATCGTTCGTCATCTGGTTGACGAACCACAAAACCTGATTAAGACGCATTTCTGGGCTCCTTC-
 TGTACGAATACTCTCGCGTTGTTGGTCTGTCTACCCTGCTGAGATTTACGAAAACTGCCACTGGTCCGGTGGT-
 CATGAAGGCCGAGGCGTCTGCTGTTGATGGGCGAAAACGTGAAATCCATCAGCATTGTAGTTCTACGTTG-
 GCAACCGTGCCAATGAACTGCTGATCAACGTCAAATCCACCAGAACCGCATCGACGAGATTACGAATAAAATG
 AACTACTACTGGACCCTGGTTGAGCCGGGCGATAAAGAACAGCTGTCCAGCGTGTCTCTTTTCGAACGTATGA
 ACACGCAGTTTACCGCTGTAGGCAAAAGCACCCAGGCTGCCATCGACCAGATCAACGGTAAAATCGATCTGTG
 GTCCTATAATGCCGAGCTGTGTTGCCCTGGAAAAATCAGCACACTATTGACCTGACTGATAGCGAAATGAA-
 CAAGGAGTTCTCTGAAGTGAAGGTGCTTGGGACCTGTTCTGTTGGAACGTCTGTCTGGCGCGATGGACGAACTG
 CACAACGAAATCCTGGAAGTGGATGAGAACTGTCTACTCACACGTTATCAACGCGAAAACGCTCCGGGTG
 GCCCGTACAAAATGTGGTGGACTACATGGTGCAGAAGAACCTGAACTCCCTGAGCGAGCTGGAAGTAAAA
 CTTTCTCCTGACTCAGGCTGCCCTGCTGAACGAAAATACAACGGCATCAATTACGGACACCATCAAATATGGT
 AACGGTGTGGATCGGTCCGGTGTGACGTGTTCTGTTATCCGCACGCTGCTGATGAACGAGCTGGGTGTTCCGT
 CCACCTGGGCACCAAGCTGGTGGATTCTGTAGTCTTGGAGCAAAGTAATCGAGGGCTGGAGCAACCCGAA-
 GATTCTGTTTATTGAAGAAGGCAAAGGTGTAACCCTGCTGCTGCCGAACCGAATGGACCTACCCGCGCCTGA
 ACGTTGAAACTGATACTGCGGAAATTCGTTATGGTGAAGCGTATACCGACACCTATCACAGCTACGTAACAAA-
 GAGTGGACCTATATCGGTGTTGATGGTCCGATAACAACGCTCTGCTGAAAGGCGGTCTGGAACCTATCAACT
 TCCAAACCGCAGCGGACCAGGCGCGTATCTCTAAGCAGTACACGCTGCCACGCAGAAAATCAACGAAGCTGG
 CCGTCTGACCGAGTGGACTTCTTCAAATGTTATGGAAGAACGTAACGTTCTGCAGCCGTTCTCCGTAGATTCTCA
 GACCGCAATGGTTCTGGTAAACGCTATCGTTTTCAAGTAAGGATCC.

2.1.2. QconCAT 2

CATATGGACGATGACGA-
 CAAGCATGTCAAAGTGGTGAACGAAGTACTGAATTCGAAAAACTTGCCTGCGTTTCGAAAAACTGGGCGAA-
 TACGGTTCCAGAACGCACTGATCGTTCGTTACACCCGTAAGTAAACACCTGGTGGATGAACCTCAAACCT
 GATCAAACAGAAGTCCCGTCCCAATCAAAGTAGTGGGTCTGTCTACCTGCCGAGATTTACGAAAAAATGG
 AAAAGCGTCCGGTGAAGCTGCCTCTGGTCCGGGTCACGAAGGCGCTGGTGTGTTGTTGGTATGGGCGAGA
 ACGTGAAGGTTGGAAGTTGTTAAATCTATTAGCATCGTTGGCTCTTACGTAGGTAACCGCGCCGACACTCGT
 GATCTGAAGAGCACCCAGAATGCCATCGATGAAATCACCAACAAAGTCAACTCTCGGAAGGTCGTATGAACTA
 CTATTGGACCCTGGTTGAACCGGGCGACAAGATTACCTTTCGTGAAGTGCAGCAACAGCTGTCTTCTGTGTCCA
 GCTTCGAACGTTTTGAAATCCGTGAATGCCGACGTTCTTCTGACGCAGGCTGACTGCTGAACGACAAAACAT
 TCCAAACCGTACTGAAATACAACGGTATCATCACTGATACCATTAAATCTGGCGCAGCTTTAAATACGGTAAC
 GCGTCTGGATCGGTCTACGAAATCTGACTGAAATCCACCAGGCGGCCATTGATCAGATCAACGGCAAAC
 GAACCGTGATACAAAATCGATCTGTGGTCTACAACGCGAAGTCTGTTGGCCCTGAAAACCAACACACCA
 TCGACCTGACCGACTCTGAAATGAACAAACTGTTGAGCGTATTGAAAAAGAATTTCCGAGGTGGAGGGTCC
 CATCCAAGATCGTCGCCCGTACCGCACTCTGCTGATGAATGAACTGGGTGTTCCATTCCACCTGGGTACAAA-
 CAGGTTTCCGCAACGGCCGCTGTTGACAGCGTAGTAAGCTGGTCTAAAGAAATCCTGCGTACCTTCAAAGT
 TATCGAAGGTTGGTCTAACCCGAAATCAAATGCTGCAGCGCTGTCTGGTGGATGGACGAACTGCACAAC
 GAGATCCTGGAAGTGGATGAGAAAGTTGACGACCGTACATCCGCTGTCCACCCACAATGTAATTAACGCA-
 GAAAACGCTCCGGGCGGTCCGTACAAAATGGTACTCGTCCGGTCTGATTGTGGTTGACTATATGGTACAGA
 AGTCTGGCAAAGCAAAAGGCGTTACGCTGCTGCCGGAACCGAATGGACTATCCCGCTGCTCCTGT
 CGTTTTCTCAAGCTGAACGTTGAAACCGACACGGCGGAAATCTGCTGATGTGTAAGTGAATATGGCGAAG
 CGTATACCGATACTTACCATTCTTACGCTAACAAGATCTGCGCCTGTATCGTGGCGGCTGGAACCGATTAAAT
 TCCAGACTGCGGCGGATCAGGCTCGTGTGAGCTGATCCGTAGCCTGAAAATCTCCAGGCACTACACGCTGCTCA
 CGCTGAAATCAACGAAGCGGGCCGTGAGGTAGTTCGTTTCGAGAACTGACCGAATGGACCAGCAGCAATGTT
 ATGGAGGAGCGTAAAATCAAATAAGGATCC.

2.1.3. *QconCAT 3 and 4*

CATATGGATGACGACGA-

CAAAGCAAGCGGTAAACTGGTGAACGAGCTGACTGAGTTCGCTAAAGCGAGCGGTAAGCTGGGTGAATACGG
 TTTCCAGAACGCACTGATCGTACGTGCTTCTGGTAAACACCTGGTCGACGAGCCTCAAACCTGATTAAAGCTA
 GCGGCAAAGACGCCTTCTGGGTAGCTTCTGTACGAGTACAGCCGTGCTTCTGGCAAAGTAGTAGGTCTGAG
 CACTCTGCCAGAAATCTACGAGAAAGCGTCTGGTAAAGCTGCCTCTGGTCGGTGGTCATGAAGGTGCTGGTGTA
 GTAGTAGGTATGGGTGAGAACGTGAAAGCATCCGGTAAAAGCATCAGCATCGTTCGGTTCTACGTTCGGTAACC
 GTGCAAGCGGTAAAGCTAACGAGCTGCTGATCAACGTCAAGGCAAGCGGCAAATCTACCCAGAACGCAATCG
 ACGAGACTACTAACAAAGCATCTGGTAAAATGAACTACTACTGGACCCTGGTCGAGCCGGGCGACAAAGCCAG
 CGTAAGGAGCAGCTGCTTCTGTGCTTCTTCTCGAGCGTGATCTGGTAAAGATGAACACCCAGTTCACGGCAG
 TGGGTAAAGCATCCGGCAAGACTTCTTCTGACTCAGGGTGACTGCTGAACGATAAAGCTTCCGGTAAATA-
 CAACGGCATCATCACGGACACCATCAAAGCGTCTGGCAAGTACGGCAACGGTGTGTGGATCGGTCTGCTTCC
 GGTAAGGGTGTGTTTTCTGTGATCCGTGCATCTGGCAAATCCACTCAGGCGCAATCGACCAAATCAACGGTAA
 GGCTTCCGGCAAGATCGACCTGTGGTCTTACAACGCTGAACTGCTGGTGTCTGGAAAACCAGCATACTATCG
 ACCTGACCGACTCTGAAATGAACAAAGCCTCCGGTAAAGAATTCTCCGAAGTGAAGGCCGCGCTCTGGTAA
 ATGGGACCTGTTTGTGGAACCGCCTCTGGTAAAGACTCTGCTGATGAACGAACTGGGTGTGCCATTTACCTGG
 GTACGAAAGCATCTGGCAAGCTGGTGGATTCTGTGGTATCCTGGTCTAAAGCCTCTGGTAAAGTTATCGAAGGC
 TGGTCCAACCCGAAAGCCAGCGGCAAGATCCTGTTTATTGAAGAAGGTAAGCGTCCGGTAAACTGTCCGGCG
 CGATGGACGAACTGCACAACGAAATCTGGAAGTGGATGAAAAGCTTCTGGCAAATGTCACCCACAACGT
 TATTAACGCGGAAAACGCCCGGCGGCAAGCTGCAAAAGCTAGCGGTAAGCTTCTGGTAAAGTATTGTTGTTGAG
 AAGCTTCCGGCAAAAACCTGAACAGCCTGTCGGAAGTGAAGCTTAAAGCTCCGGCAAAGGCTTACCTGCT
 GCTGCCGGAACCGAATGGACCTACCCGCGTGCCAGCGGCAAACTGAATGTTGAAACCGATACCGCTGAAAT
 CGCGCCAGCGGTAATATGGCGAAGCTTATACCGATACTATCACTCTATCGGAACAAAGCGAGCGGCAAAGA
 ATGGACCTATATTGGCGTAGATGGCCCGGATAACAACGCGCTGCTGAAAGCCTCTGGCAAAGGCCGCTGGAA
 CCGATTAATTTTACAGACCGCGGTGATCAGGCTCGTGCAGCGGTAAAATTTCTCAGGCGGTTATGCGGCGCA
 CGCGGAAATTAATGAAGCGGCGCTGCTGCGCAAAGTACCGAATGACGCTTCTAATGTTATGGAAGAA
 CGCGCATCCGGCAAAAATGTTCTGCAACCGTCTAGCGTTGATTCCAGACCGCGATGGTTCTGGTAAATGCGAT
 TGTTTTCAAAGCGTCCGGCAAATAAGGATCC.

2.1.4. *QconCAT 5*

CATATGGCTGGTCTGTCGCTCTGGTAAACTGGGTGAGTACGGTTTTTCCAGAACCGCGTATCGTACGTGCGTCT
 GGTAAGTAGTTCGGTCTGTCTACCCTCCCGGAGATCTACGAAAAAGCGTCCGGTAAAGAGGTCCTGGTCTGTG
 GGTATTACCACCCGCTACTTCTGAGATCAGCAGTCTCTGTACCAGAACGAGACGCTTACGTATTCTGTTGG
 CTCTTCTCGTGGTCTGGCAAATCGACTGTGGTCTTACAACCGCGAAGTCTGGTTGCCCTGGAAAACCAGC
 AACTATCGACCTGACCGACTCCGAAATGAACAAAGCGTCCGGTAAACTGTCTGGTGGATGGACGAACTGCA-
 CAACGAAATCCTGGAAGTGGACGAGAAAGCCAGCGGTAACCTTCTTCTGACTCAGGGTGGCTGCTGAAC
 GACAAAGCTTCTGGCAAACCTGCTGATGAACGAACTGGGTGTTCCGTTTACCTCGGTACCAAAGCGTCTGG
 TAAAGGTGTTACCTGCTGCTGCCGGAACCGGAATGGACTTATCCACGTGCCTCTGGTAAAGTGGTCTGGAAC
 CGATCAACTTTCAGACGCGCGGATCAGGCAGCTGTTCTGGTAAATCTCTCAGGCTTTCACGCGCGCA
 CGCAGAAATCAACGAAGCAGGTCGTGCTTCTGGCAAAGTGAACGTTGAAACCGACACCGCGGAAATCCGTGCC
 TCTGGTAAACTGGTTGACAGCGTTGTTTCTTGGTCCAAAGCGTCCGGTAAATACAACGGTATCATACCGACACC
 ATCAAAGCCTCTGGTAAATTCACCTCCTCTGCCAACGGTGTACGACCCACTACGTATCTCAGATCGGTGTTTC
 CCGATCAGACCGAAGACGGTGGTCTGCCGAGTCTGGTCTGCTTCTGGTAAATCTACCCAGGCGCGGATTG
 ACCAGATCAACGGTAAAGCGTCCGGCAAATCTACGCAGAACCGATCGACGAGATCACCAACAAAGCCTCTGG
 TAAACTGCCGCTGGTAGGTGGTACGAAGGTGAGGTGTTGATGGGTGAGAACGTGAAAGCGA
 GCGGTAAACTGGTAAACGAACTGACGGAGTTCGCAAAGCCTCTGGTAAAACCTGAACAGCCTCAGCGAAC
 TGGAAAGTAAAGCCTCTGGCAAATACGGTAACGGTGTGGATCGGTCTGACGGTAAATCTGGTTACAG
 CGTATCTTCTGTTGAGGTTAAAGCCTCCGGTAAATACGGTGAAGCTACAGGATACCCACTACCCTTACG
 CCAAAGCGAGCGGTAACCTGACCAATGACGAGCTTAACTGATGGAGGAACGTGCTCTGGTAAAGACG
 CTTTCTGGGCTCCTTCTGTACGAATACTCTGTCGCTCTGGTAAATCTATCTATCTGTCGGCTCCTACGTAGG
 TAACCGTGGTCTGGTAAATGAACTACTACTGGACCCTGGTTGAACCGGGTGACAAAGCTTCCGGTAAATCTC

AGCAGGCGGTTATCCCGAACATCGGTTTTCTGTCACGTGCTTCTGGCAAACCTGAACTGGCTGACCCACCTGAAC
 TTCAAAGCCTCCGGTAAAATGAACACCCAGTTACAGGCGGTTGGTAAAGCGTCTGGTAAAGCCAACGAACTGC
 TGATCAACGTGAAAGCCTCCGGTAAACACCTCGTAGACGAACCGCAGAACCTGATCAAAGCGTCTGGCAAAA
 ACGTACTCCAGCCGTTCTGTTGACTCTCAGACCGCTATGGTTCTGGTCAACGCGATCGTATTCAAAGCCAGC
 GGTAAAGGTAACCTGCCCCGCTGATCATTCTGCTCTGTTAAAGGTTGGGCTTTTGACGACGTAACGACGT
 TTGGATGGGTCTGCTGCTGGTAAAGGCGACGTATTCTGTTATCCGTGCGTCTGGTAAAGCGGACACCATCTCTT
 CTCAGATCGAACTGGCCGTTCTGCTGTCTAACGAGGGTATCATCAACTCCGAAGACGAGCACCTGCTGGCTCTG
 GAACGTGCCTCTGGTAAACTGGCAGCGGCCCTGGAACACCACCACCACCACCTAATAGGGATCC.

2.2. Peptide Sequences

2.2.1. QconCAT 1-4

Protein	Peptides (*sequence omitted in QconCAT 2)
BSA – Bovine Serum Albumin	LVNELTEFAK LGEYGFQNALIVR HLVDEPQNLIK DAFLGSFLYEYSR*
ADH – Alcohol Dehydrogenase (<i>Saccharomyces cerevisiae</i>)	VVGLSTLPEIYK LPLVGGHEGAGVVVGMGENVK SISIVGSYVGNR ANELLINVK*
H1 – Hemagglutinin (H1N1)	STQNAIDEITNK MNYWTLVEPGDK EQLSSVSSFER MNTQFTAVGK*
N1 – Neuraminidase A/California (H1N1)	TFFLTQGALLNDK YNGIITDTIK YGNVWIGR GDVFFVIR*
H3 – Hemagglutinin A/Victoria (H3N2)	STQAAIDQINGK IDLWSYNAELLVALENQHTIDLTLDSEMNK EFSEVEGR WDLFVER*
N2 – Neuraminidase A/Victoria (H3N2)	TLLMNELGVPFHLGTK LVDSVVVSWSK VIEGWSNPK ILFIEEGK*
HB – Hemagglutinin B/Brisbane	LSGAMDELHNEILELDEK LSTHNVINAENAPGGPYK IVVDYMVQK NLNSLSELEVK*
NB – Neuraminidase B/Brisbane	GVTLLEPEPEWYPR LNVETDTAEIR YGEAYTDTYHSYANK EWTYIGVDGPDNNALLK*
Oval – Ovalbumin (<i>Gallus gallus</i>)	GGLEPINFQTAADQAR ISQAVHAAHAEINEAGR LTEWTSSNVMEER NVLQPSVSDSQTAMVLVNAIVFK*

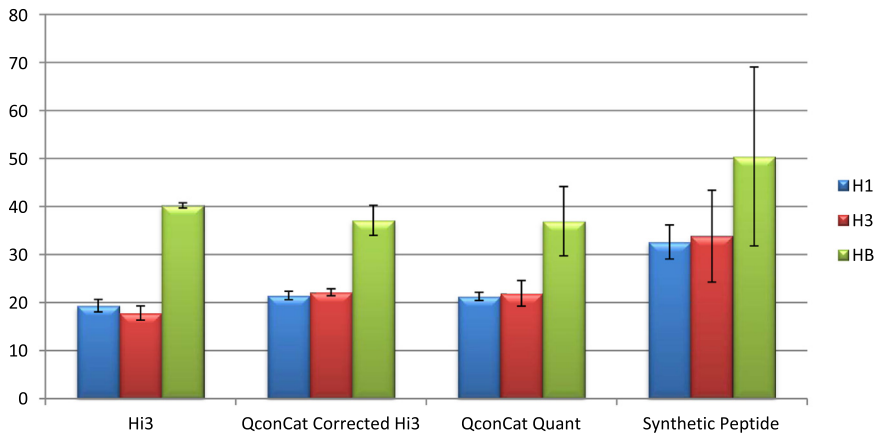
2.2.2. QconCAT 5 and SpikeTides

Protein	Peptides (sequence unique to QconCAT 5) (*sequences obtained as SpikeTides)
BSA – Bovine Serum Albumin	LGEYGFQNALIVR* LVNELTEFAK* DAFLGSFLYEYSR* HLVDEPQNLIK
ADH – Alcohol Dehydrogenase (Saccharomyces cerevisiae)	VVGLSTLPEIYEK LPLVGGHEGAGVVVGMGENVK SISIVGSYVGNR ANELLINVK
H1 – Hemagglutinin (H1N1)	EVLVLWGIHPSTSadQQSLYQNADAYVFGSSR* STQNAIDEITNK* MNYWTLVEPGDK* MNTQFTAVGK
N1 – Neuraminidase A/California (H1N1)	TFFLTQGALLNDK YNGIITDTIK YGNQVWIGR GDVVFVIR
H3 – Hemagglutinin A/Victoria (H3N2)	IDLWSYNAELLVALENQHTIDLTDSEMNK* STQAaidQINGK* SQqAVIPNIGFRPR* LNWLTHLNFK
N2 – Neuraminidase A/Victoria (H3N2)	TLLMNELGVPFHLGK LVDSVVSWSK SGYSGIFSVGK GWAFDDGNDVWMGR
HB – Hemagglutinin B/Brisbane	LSGAMDELHNEILELDEK* FTSSANGVTTHYVSQIGGFPDQTEDGGLPQSGR* NLNSLSELVK* ADTISSQIELAVLLSNEGIINSEDEHLLALER
NB – Neuraminidase B/Brisbane	GVTLLLPEPEWYPR LNVETDTAEIR YGEAYTDYHSYAK GNSAPLIIR
Oval – Ovalbumin (Gallus gallus)	GGLEPINFQTAADQAR ISQAVHAAHAEINEAGR LTEWTSSNVMEER NVLQPSSVDSQTAMVLVNAIVFK

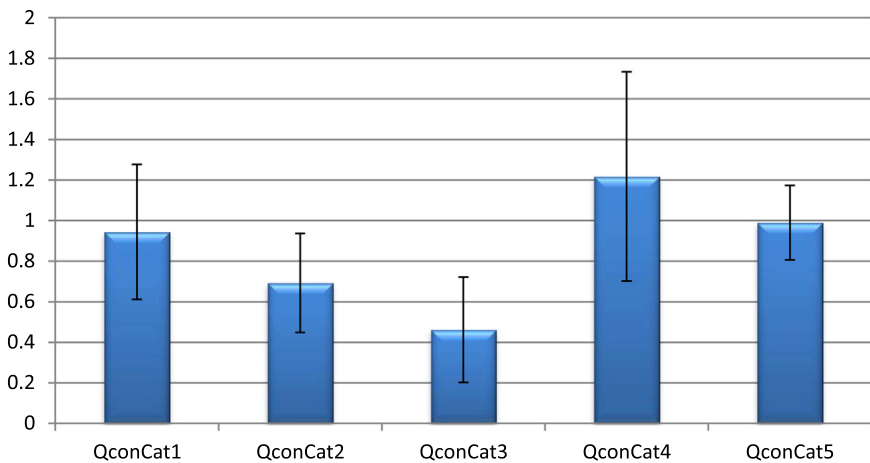
QconCAT final sequence where using the spacer peptide **ASGK** :

**MAGRA ASGKBSA-1 ASGKADH-1 ASGKH1-1 ASGKH3-1 ASGKHB-1 ASGKN1-1
 ASGKN2-1 ASGKNB-1 ASGKOV-1 ASGKOV-2 ASGK NB-2 ASGKN2-2 ASGKN1-2
 ASGKHB-2 ASGKH3-2 ASGKH1-2 ASGKADH-2 ASGKBSA-2 ASGKHB-3 ASGKN1-3
 ASGKN2-3 ASGKNB-3 ASGKOV-3 ASGKBSA-3 ASGKADH-3 ASGKH1-3
 ASGKH3-3 ASGKH3-4 ASGKH1-4 ASGKADH-4 ASGKBSA-4 ASGKOV-4 ASGKNB-4
 ASGKN2-4 ASGKN1-4 ASGKHB-4 ASGKLAAALEHHHHHH**

Hemagglutinin ($\mu\text{g/ml}$) in trivalent influenza vaccine by quantitative method



Ratio of average "Hi3" values from all proteins represented in QconCat relative to BSA standard.



FASTA Protein Database used: 2015_2016_FluQuant.fasta

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Transparency document. Supplementary material

Transparency data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.dib.2016.08.035>.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.dib.2016.08.035>.

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